Serial No. 08/211,800

Examiner: D. Rees

Filed: April 14, 1994

Group Art Unit: 1807

SCHEDULE A2

CLAIM AMENDMENTS

Please cancel claims 1 to 31 and add the following claims 32 to 47.

A binding assay process for determining the concentration of one or more analytes in a liquid sample

using a capture binding agent having binding sites specific for each analyte expected to be present in the sample and a developing binding material capable of binding to bound analyte, to binding sites of the capture binding agent occupied by bound analyte or to binding sites remaining unoccupied by the analyte,

the capture binding agent for a given analyte being immobilized at high density on a support in the form of one or more microspots each having an area less than 1 mm^2 , and wherein

labelled microspheres having a diameter less than 5 µm being used in the assay in relation to the developing binding material, so that the strength of the signal from the label is representative of the fractional occupancy of the binding sites of the capture binding agent, thereby allowing the concentration of the analyte to be determined.

1. The process of claim 32, wherein the capture binding

agent specific for a given analyte is used in a small amount that binds less than 5% of the analyte in the sample.

- 3 M. The process of claim M, wherein less than 0.1V/K moles of a capture agent specific for a given analyte are used, where V is the sample volume in liters and K is the effective affinity constant of the capture binding agent for the analyte under the conditions of the assay.
- The process of claim $3\mathbb{Z}$, wherein the capture binding agent is immobilized at a surface density of 10,000 to 50,000 molecules/ μ m².
- 5 36. The process of claim $\frac{1}{2}$, wherein the microspots have an area less than 100 μ m².
- The process of claim 32, wherein the concentration of a plurality of analytes is determined in the same operation using a plurality of different capture binding agents, each capture binding agent having binding sites specific for a given analyte in the sample.
- The process of claim 37, wherein one or more developing binding materials are used in the assay, the developing binding materials and the labelled microspheres being capable of binding to each other, so that the same label is used in relation to the developing binding materials, with the microspots containing different capture binding agent

being distinguished apart by their location on the support.

- The process of claim 38, wherein one of the microspheres and the developing binding materials is conjugated to biotin and the other to avidin or streptavidin.
- 9 46. The process of claim 32; wherein the label is contained within the microspheres.
- 10 A. The process of claim 32, wherein the label is a fluorescent label.
- The process of claim 32, wherein the microspheres are blocked to minimize their non-specific interactions with other materials.
- 12 13. The process of claim 32, wherein the capture binding agent and the developing binding material are antibodies.
- 13 4. The process of claim 32, wherein the analyte is a nucleic acid sequence, the capture binding agent is an oligonucleotide sequence capable of binding the analyte and the developing binding material is oligonucleotide sequence or an antibody capable of hybridizing to the analyte.
- A binding assay process for detecting the presence of one or more target nucleic acid sequences in a liquid sample

- 19 using one or more capture binding agents comprising an oligonucleotide sequence capable of hybridizing to a given target nucleic acid sequence and a developing binding material comprising an oligonucleotide sequence capable of hybridizing to the bound target nucleic acid sequence, the capture binding agent for a given target nucleic acid sequence being immobilized at high density in the form of one or more microspots each having an area less than 1 mm2, and wherein labelled microspheres having a diameter less than 5 μm are used in the assay in relation to the developing binding material, so that the signal from the label indicates the presence of the target nucleic acid sequence, thereby allowing the presence of said one or more target nucleic acid sequences to be detected. 46. A kit for determining the concentration of one or more analytes in a liquid sample, the kit comprising: one or more capture binding agents, each capture binding agent having binding sites specific for a given analyte expected to be present in the sample, wherein the capture binding agents are immobilized at high density on a support in the form of one or more microspots, each microspot having an area less than 1 mm²; and, one or more developing binding materials, each developing binding material being capable of binding to a given bound analyte, to binding sites of a given capture

binding agent occupied by bound analyte or to binding sites of

a given capture binding agent remaining unoccupied by the analyte;

wherein labelled microspheres having a diameter less than 5 μm are used in the assay in relation to the developing binding material, so that the strength of the signal from the label is representative of the fractional occupancy of the binding sites of a given capture binding agent, thereby allowing the concentration of the analyte to be determined.

No

The kit of claim 46, further comprising standards containing known amounts or concentrations of analyte.